

FIG. 1

Context-Sensitive Parallel Optimization

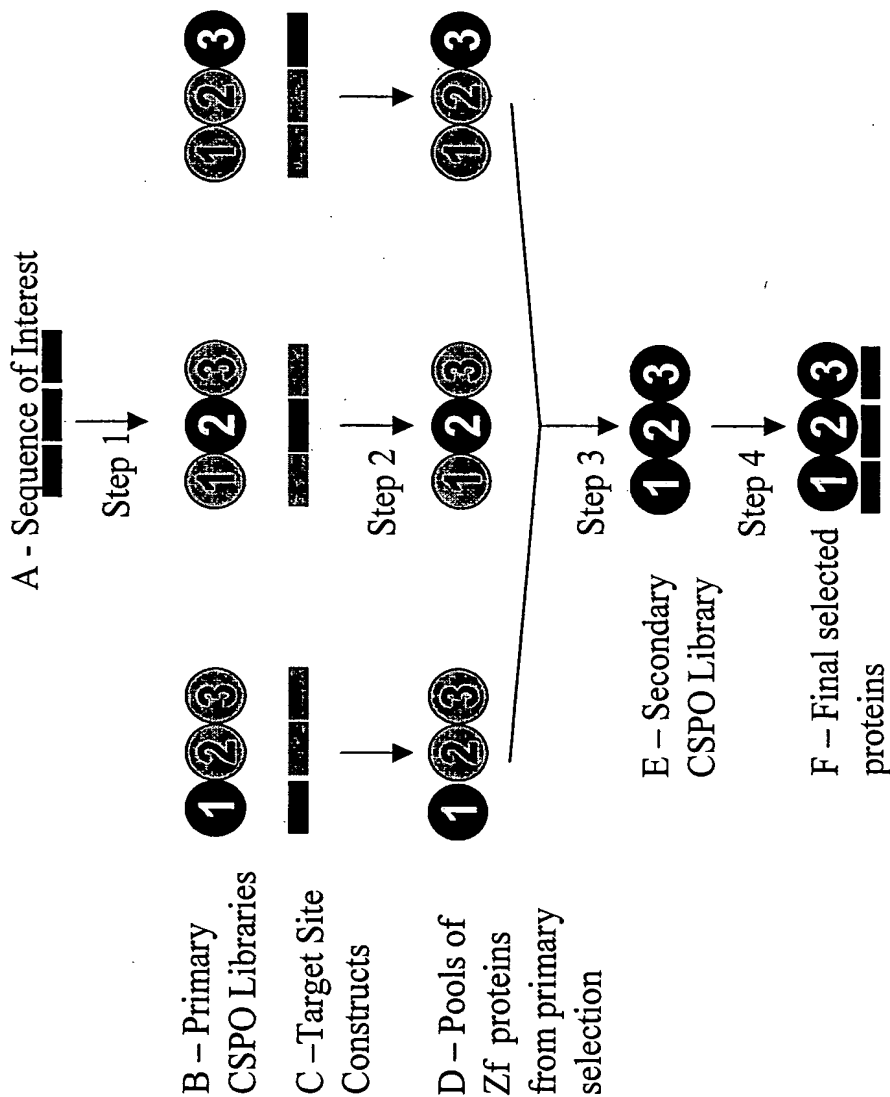


FIG. 2

Construction of Randomly Recombined Libraries

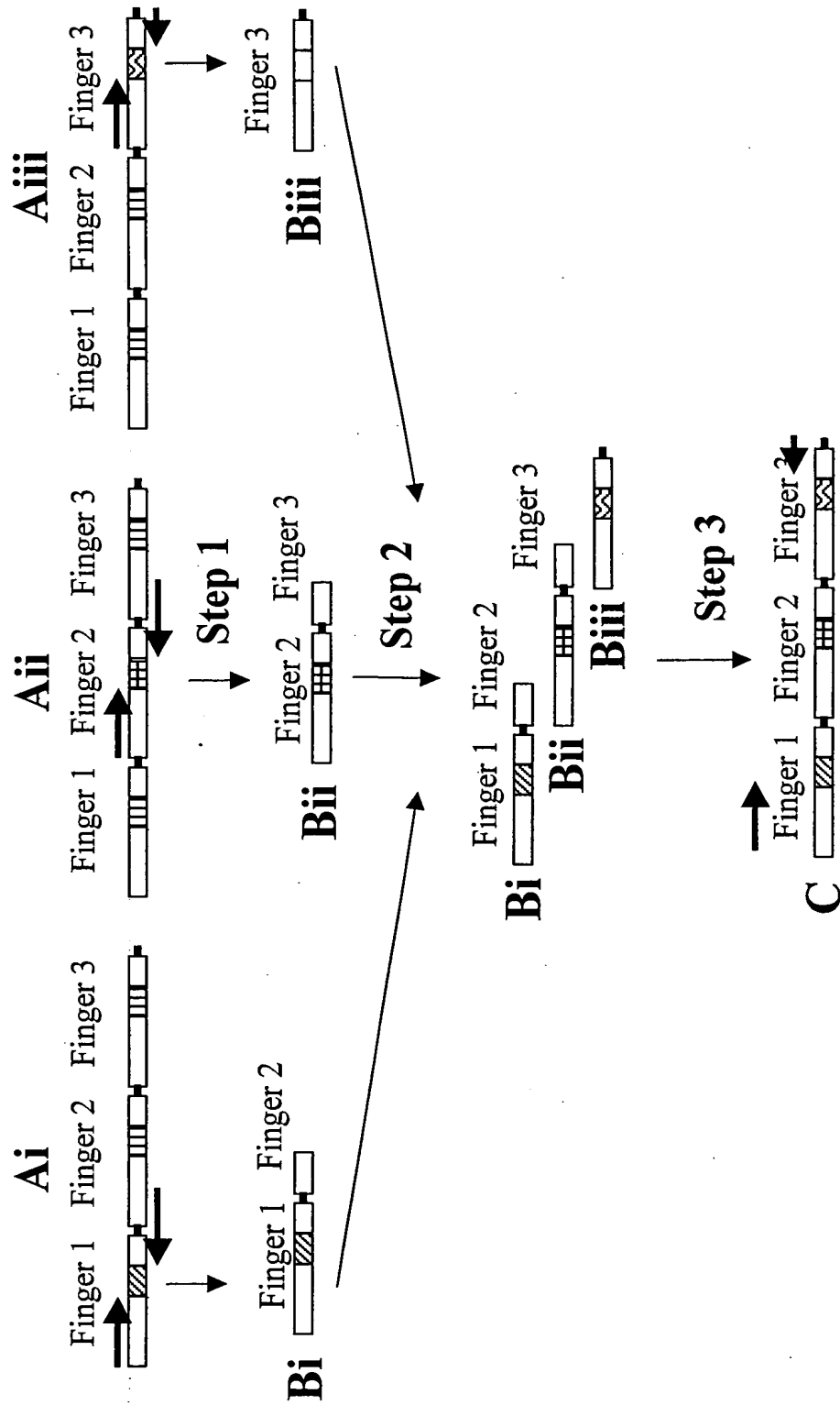


FIG. 3
Quantifying Affinity of ZFPs

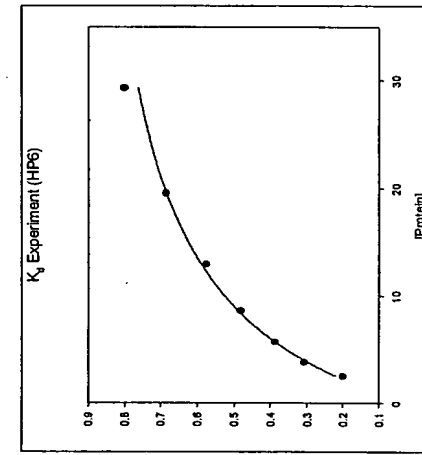
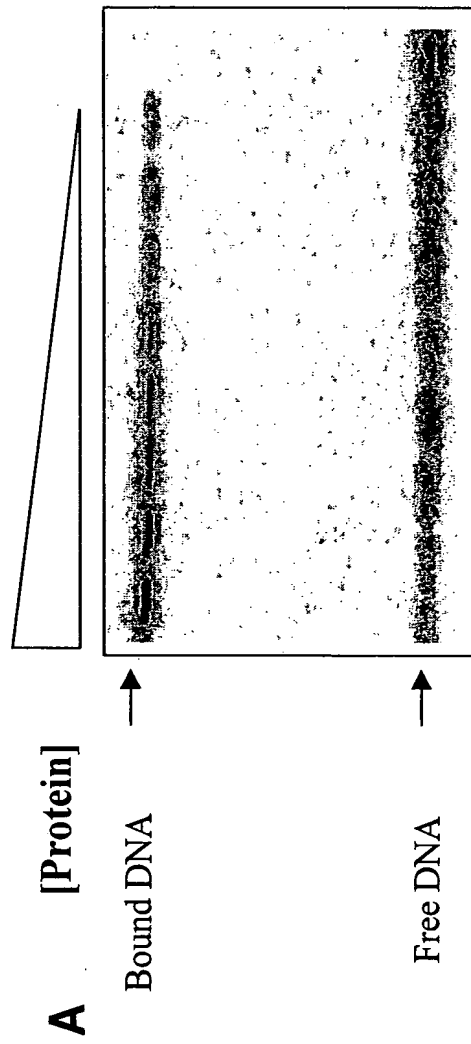


FIG. 4
Characterizing Specificity of ZFPs

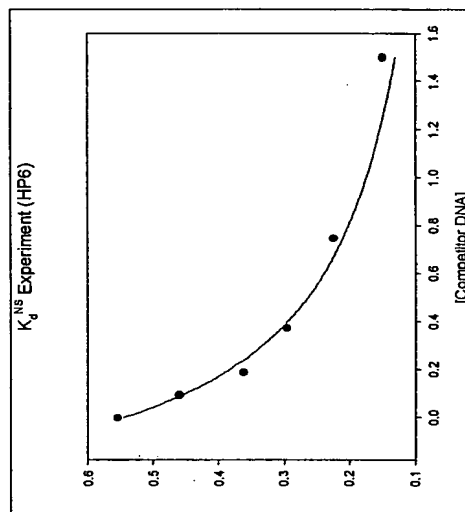
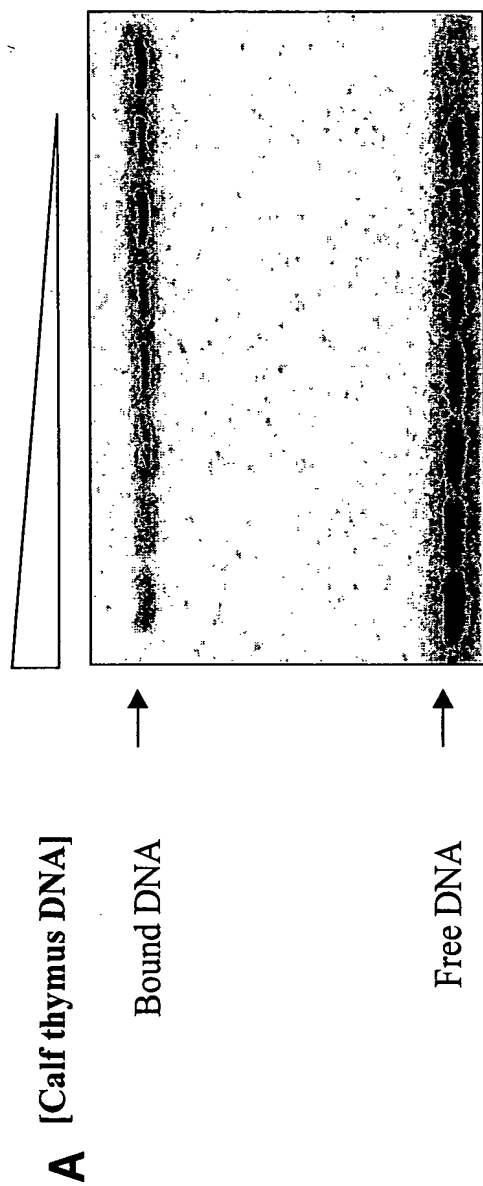


FIG. 5
Validating Context-Sensitive Parallel Optimization

A	i)	BCR-ABL	5'GCAGAGGCC ^{3'}
	ii)	erb-B2	5'GCCGCAGTG ^{3'}
	iii)	HIV promoter	5'GATGCTGCA ^{3'}
B	i)	BCR-ABL	DRSSTR QGGNVR QAA ^{TQR*}
	ii)	erb-B2	RKDSVR QSG ^{DDRR} DCRDAR [*]
	iii)	HIV promoter	ASADTR NRSDSR TSS ^{NKK#}

FIG. 6

Selections for the BCR-ABL site

CCG AAG ACG ← DNA target site

F1 F2 F3

-1 12356 -1 12356 -1 12356

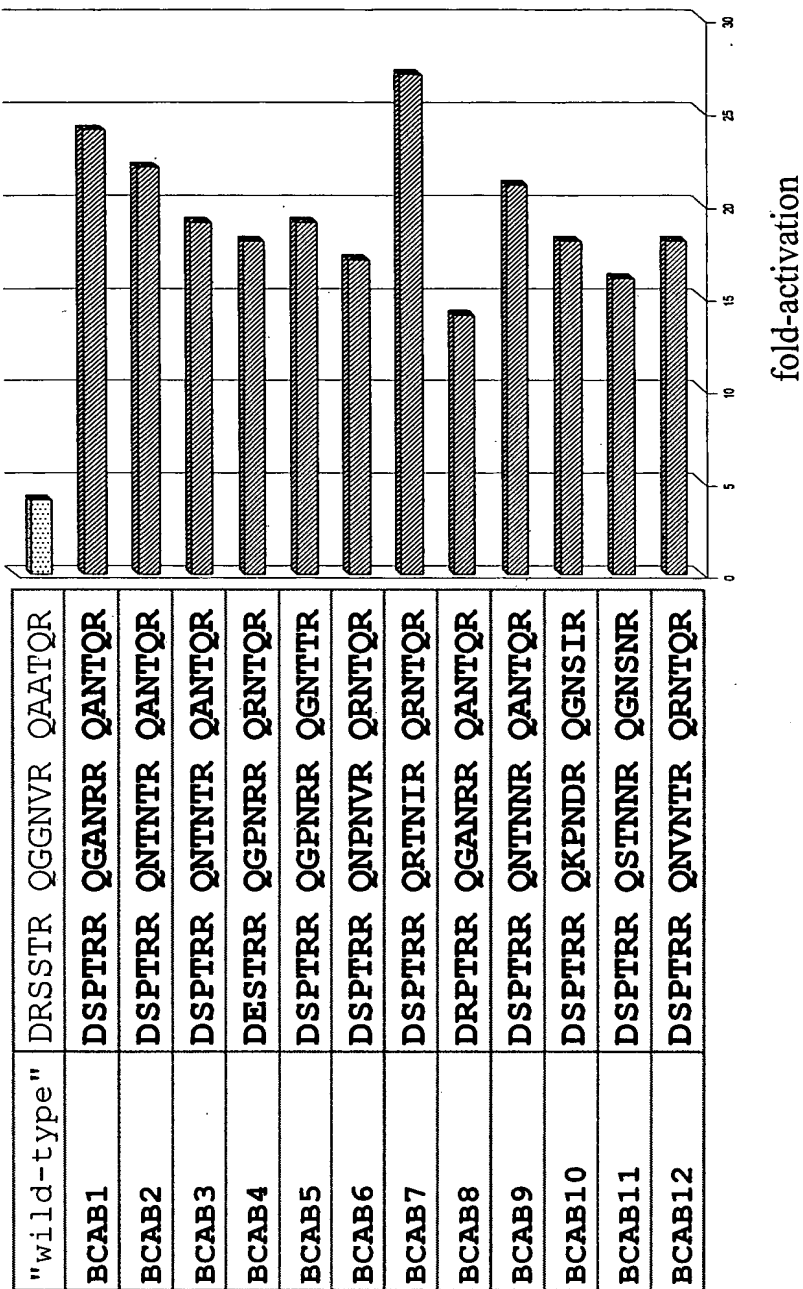


FIG. 7

In vitro characterization of BCR-ABL ZFPs

Protein	Sequence	K_d^{spec} (pM)	$K_d^{non-spec}$ (nM)	Specificity ratio	# of DNA bases specified
"wt"	DRSSTR QGGNVR QAATQR	28 (± 3.9)	55 (± 12)	1,980	~5.5
BCAB 1	DSPTRR QGANRR QANTQR	78 (± 13)	2100 (± 270)	27,000	~7.4
BCAB 7	DSPTRR QRTNIR QRNTQR	60 (± 8.5)	1300 (± 97)	23,000	~7.2
Zif268		8.1 (± 1.8)	1000 (± 120)	130,000	~8.5

10/532258

FIG. 8

Selections for the erb-B2 site

GTG ACG CCG ← DNA target site
F1 F2 F3

-1 12356-1 12356-1 12356

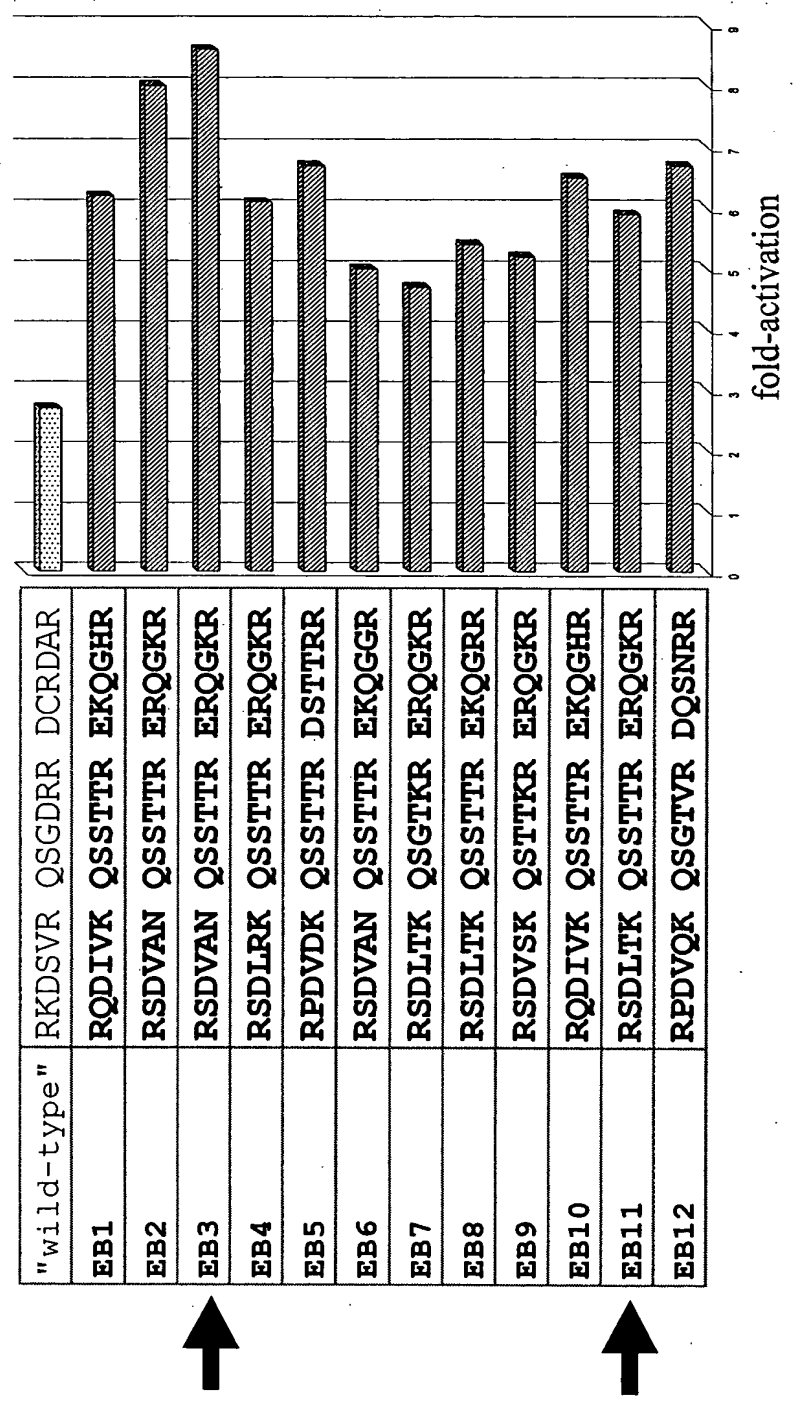


FIG. 9

In vitro characterization of erb-B2 ZFPs

Protein	Sequence	K_d^{spec} (pM)	$K_d^{non-spec}$ (nM)	Specificity ratio	# of DNA bases specified
"wt"	RKDSVR QSGDRR DCRDAR	150 (± 23)	1000 (± 120)	6,700	~6.4
EB 3	RSDVAN QSSSTR ERQGKR	31 (± 3.1)	1100 (± 15)	35,000	~7.5
EB 11	RSDLTK QSSSTR ERQGKR	65 (± 3.9)	1100 (± 81)	17,000	~7.0
Zif268		8.1 (± 1.8)	1000 (± 120)	130,000	~8.5

10/532258

FIG. 10

Selections for the HIV promoter site

ACG TCG TAG ← DNA target site

F1 F2 F3

-1 12356 -1 12356 -1 12356

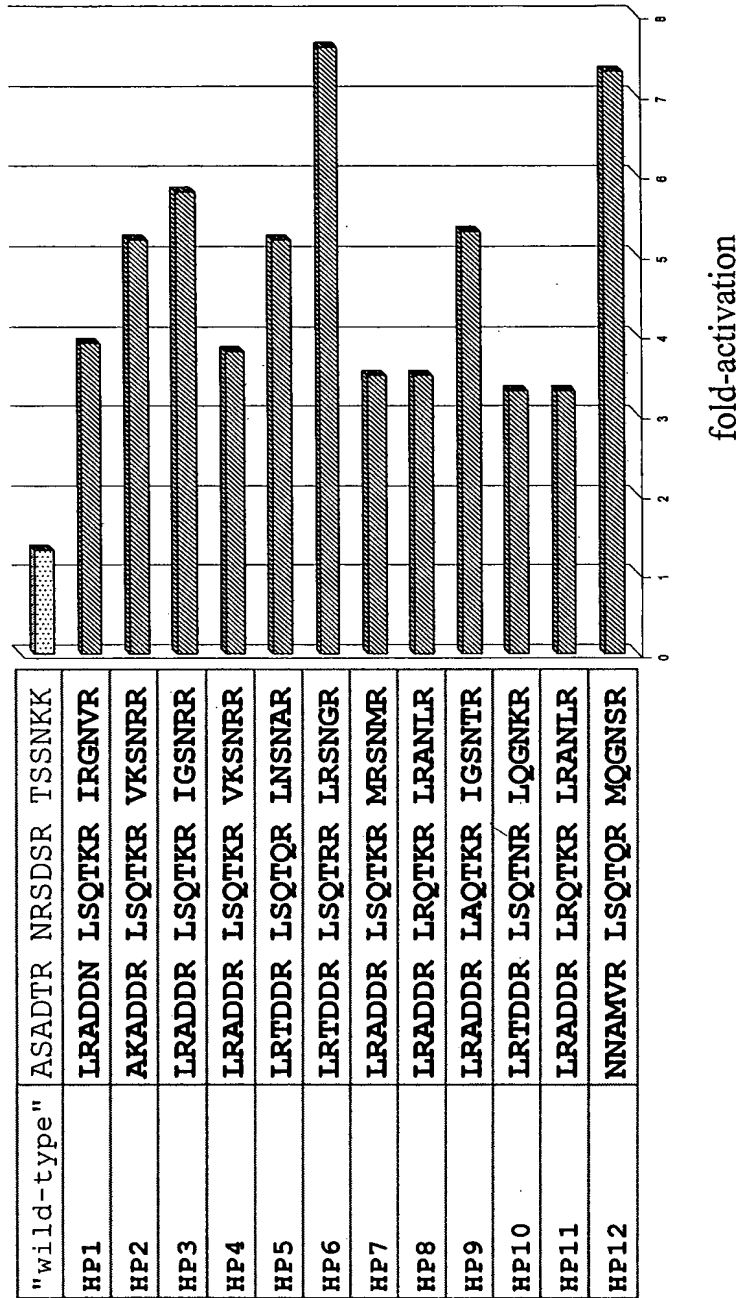


FIG. 11

In vitro characterization of HIV Promoter ZFPs

Protein	Sequence	K_d^{spec} (pM)	$K_d^{non-spec}$ (nM)	Specificity ratio	# of DNA bases specified
"wt"	ASADTR NRSDSR TSSNKK	Unable to calculate (does not bind in vitro)			
HP6	LRTDDR LSQTRR LRSNGR	9.3 (± 1.2)	820 (± 74)	87,000	~8.2
HP12	NNAMVR LSQTQR MQGNSR	9.3 (± 0.39)	180 (± 8.8)	19,000	~7.1
Zif268		8.1 (± 1.8)	1000 (± 120)	130,000	~8.5

10/532258